DETECTION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

STATEMENT OF PRIORITY

The present invention claims the benefit, under 35 U.S.C. §119(e), of U.S. Provisional Application No. 61/008,680, filed Dec. 21, 2007, the entire contents of which are incorporated by reference herein.

FIELD OF THE INVENTION

The present invention relates to molecular detection of methicillin-resistant *Staphylococcus aureus* (MRSA). More particularly, the present invention relates to an improved detection of MRSA that reduces false positive results.

BACKGROUND OF THE INVENTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial but also community acquired pathogen that can cause serious infections such as surgical wound infections, pneumonia, endocarditis and septicemia. Resistance to methicillin is due to the presence of the mecA gene that encodes a modified Penicillin-Binding protein, PBP2a or PBP2', with reduced affinity for B-lactam drugs. The mecA gene is carried by a cassette named the SCCmec (Staphylococcal Cassette Chromosome mec; Ito et al., 2001, Antimicrob. Agents Chemother. 45(5):1323-1336, Hiramatsu, et al., 2001, Trends Microbiol. October; 9(10):486-93), a mobile element that can be incorporated into the chromosome of *S. aureus* and other coagulase negative Staphylococci, mainly *S. epidermidis* and *S. haemolyticus*. SCCmec is characterized by the presence of terminal inverted and direct repeats, a set of site-specific recombinase genes (ccrA and ccrB), and the mecA gene complex (Ito et al., 1999, Antimicrob. Agents Chemother. 43:1449-1458; Katayama et al., 2000, Antimicrob. Agents Chemother. 44:1549-1555). The site of insertion of this mecA gene cassette SCCmec into the *Staphylococcus aureus* genome is known and the sequence conserved (Ito et al., 2001, Antimicrob. Agents Chemother. 45:1323-1336). After insertion into the *S. aureus* chromosome, the SCCmec has a left extremity junction region and a right extremity junction region (see FIG. **1**), where the SCCmec sequence is contiguous with the *S. aureus* chromosomal sequence. The nucleotide sequence of the regions surrounding the left and right boundaries of SCCmec DNA (i.e. attL and attR, respectively), as well as those of the regions around the SCCmec DNA integration site (i.e., attBscc, the bacterial chromosome attachment site for SCCmec DNA), have previously been analyzed. Sequence analysis of the integration sites revealed that attBscc is located at the 3' end of a novel open reading frame (ORF), orfX. orfX encodes a putative 159-amino acid polypeptide that exhibits sequence homology with some previously identified polypeptides of unknown function (Ito et al., 1999, Antimicrob. Agents Chemother. 43: 1449-1458). Organization of the mecA region of SCCmec has additionally been studied (Oliveira, D. C., et al., 2000, Antimicrob. Agents Chemother. 44(7):1906-1910).

MRSA can be carried by healthy people without causing any disease but these healthy carriers, when entering the hospital, can contaminate hospitalized patients. Additionally, a patient can contaminate himself, e.g., if one undergoes surgery, the risk of infection is increased. MRSA healthy carriers constitute a reservoir of MRSA and screening of these carriers must be performed to eradicate the strains by local decontamination. MRSA screening is now recognized as a major tool to reduce the prevalence of MRSA strains in the world. Typically, in an MRSA assay in a patient, a nasal swab is taken from the patient and cultured repeatedly, to determine if an MRSA strain is present. The need to culture could be obviated by an assay for identifying MRSA directly from a nasal swab. Culture identification methods typically require minimally 24 hours, and more typically 72 hours, to obtain results. New chromogenic media (having substrate(s) within the media and, typically, antibiotic (e.g., cefoxitin) to select methicillin-resistant strains) can potentially restrict this time to result to a 24-48 hour time period. However, in the case of MRSA infection, results are needed in a matter of hours, since the patient should be isolated until results are obtained. Therefore, a reliable molecular MRSA test which can provide results in a matter of 2-4 hours is highly desirable.

Amplification is a well known art, and various methods have been developed, including transcription-based amplification such as transcription-mediated amplification (TMA; U.S. Pat. Nos. 5,766,849 5,399,491; 5,480,784; 5,766,849; and 5,654,142) and nucleic acid sequence-based amplification (NASBA; 5,130,238; 5,409,818; 5,654,142; and 6,312,928), and cycling nucleic acid amplification technologies (thermocycling) such as polymerase chain reaction (PCR; U.S. Pat. Nos. 4,683,195; 4,965,188; 4,683,202) and ligase chain reaction (LCR; U.S. Pat. No. 5,792,607). Known amplification methods also include strand displacement amplification (SDA), self-sustained sequence replication (3SR), Q-β replicase, and cascade rolling circle amplification (CRCA).

Detection methods utilizing nucleic acids are also well known in the art. Nucleic acids are often labeled for various detection purposes. For example, methods described in U.S. Pat. Nos. 4,486,539 (Kourlisky); 4,411,955 (Ward); 4,882,269 (Schneider) and 4,213,893 (Carrico), illustrate preparation of labeled detection probes for detecting specific nucleic acid sequences. Probe designs for different detection methods, such as target-capture, HPA, TAQman, molecular beacons and sandwich hybridization have also been described (e.g., U.S. Pat. No. 4,486,539, and U.S. Pat. Nos. 4,751,177; 5,210,015; 5,487,972; 5,804,375; 5,994,076). Nucleic acid hybridization techniques and conditions are known to the skilled artisan and have been described for example, in Sambrook et al. Molecular Cloning A Laboratory Manual, 2nd Ed. Cold Spring Lab. Press, December 1989; U.S. Pat. Nos. 4,563,419 (Ranki) and 4,851,330 (Kohne) and in Dunn, et al., *Cell* 12, pp. 23-26 (1978) among many other publications. Probe designs for different detection methods are also known, such as target-capture, HPA, TaqMan, molecular beacons and sandwich hybridization (e.g., U.S. Pat. No. 4,486,539, and U.S. Pat. Nos. 4,751,177; 5,210,015; 5,487,972; 5,804,375; 5,994,076).

Earlier molecular methods developed to detect and identify MRSA based on the detection of the mecA gene and *S. aureus*-specific chromosomal sequences have been described. (Saito et al., 1995, J. Clin. Microbiol. 33:2498-2500; Ubukata et al., 1992, J. Clin. Microbiol. 30:1728-1733; Murakami et al., 1991, J. Clin. Microbiol. 29:2240-2244; Hiramatsu et al., 1992, Microbiol. Immunol. 36:445-453). However, positive results for the presence in a sample of both mecA gene and *S. aureus* chromosomal sequences cannot guarantee MRSA is present, since, for example, in tests based on the detection of mecA and *S. aureus* specific marker, false positives can be observed in the presence of MSSA and methicillin resistant coagulase negative *Staphylococcus* that possess the mecA gene. Furthermore, in tests based on the detection of the cassette junction only, false positives have been observed with methicillin-susceptible *S. aureus* isolates